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[8] In subsequent studies, a *Cy/Pm* strain responded similarly to selection.

[9] Reciprocal differences in crosses involving *lo-2* were first observed in these experiments and therefore are recorded here. Detailed analysis of this phenomenon will be published elsewhere.

[10] Experiments performed by Mr. S. Waltman in our laboratory indicate that the enzyme systems of the *lo-2* and *lo-3* strains differ qualitatively. Almost all of the enzyme in extracts of *lo-2* is heat-labile, while extracts from *lo-3* contain both heat-labile and heat-stable enzyme. The two strains differ also with respect to their catalytic effect on the oxidation of the substrates dopa and catechol.

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*INHIBITION BY PERIODATE OF MATING IN ESCHERICHIA COLI K-12**

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The oxidation of carbohydrates by the periodate ion is the basis of the well-known McManus-Hotchkiss histochemical procedure.[1] It also played an important role in the identification of the mucopolysaccharide receptors for the influenza virus on the surface of tissue cells.[2] In the course of a routine survey of reagents for their effect on conjugation in *Escherichia coli*,[3] it was noted that periodate inhibited the mating reaction at a concentration that left most of the cells viable. As will be shown in the present paper, this inhibition can be attributed to the alteration of a substance, presumably carbohydrate, specifically associated with the conjugal activity of *male* cells.

Materials and Methods.—The general methods and the media have been described previously.[4] The strains used, which are all derived from *E. coli K-12*, and their genetic markers are as follows:[5]

W	2324	Hfr_2, Th^-, S^r.
W	3064	F^-, T^-, L^-, Th^-, Lac^-_1, Gal^-_7, Xyl^-_1, Ara^-_1, Mal^-_1, V^r_λ, V^r_1, V^r_6, S^r.
W	3086	F^-, M^-, S^r.
W	6	F^+, M^-.
W	3876	F^-, ♀ 3, Lp^s, Lac^-_{11D3}, Mal^-_5, S^r.
W	3776	Hfr_2, Lp^s, T^-, L^-, Lac^-_1, V^r_1, V^r_6.
W	3780	Hfr_2, Lp^s, M^-Az^rS.

The strains were routinely grown overnight at 37°C in Difco "penassay" broth on a rotator, and the organisms (which numbered about 5×10^8 per ml) were washed once and resuspended at 0°C in distilled water at twenty times their original density. For periodate treatment, 0.1 ml of such a cell suspension was added to 0.4 ml of sodium metaperiodate solution made up in minimal medium without glucose.[4] The mixture was incubated for 10 minutes at 37°C, and the residual periodate was then destroyed by adding 4.5 ml of penassay broth. The treated cells were then mated with cells of the opposite mating type in penassay broth. After 15 minutes at 37°C, suitable dilutions were plated onto appropriate selected media for the estimation of recombinants. Routinely, the transfer of T^+L^+ alleles from Hfr_2 male cells into $T^-L^-F^-$ cells was studied by plating on minimal medium containing streptomycin to kill the male parental cells and also containing thiamin. The mating time of 15 minutes at 37°C was adequate for this purpose. The plates were thoroughly rubbed with a glass spreader until they were dry. This procedure has been found adequate to break up mating pairs with these strains and to reduce the background of plate recombination.

When plate recombination was encouraged (Table 2), the minimal medium was enriched 1:10 with penassay broth.

TABLE 1

EFFECT OF SODIUM PERIODATE ON MATING ABILITY OF MALE AND FEMALE STRAINS OF *Escherichia coli*

	♂* × ♀*	♂ × ♀*	♂* × ♀	♂ × ♀
Viable bacteria per 10^{-6} ml ♂:	53	99	46	84
♀:	98	120	148	120
Recombinants per 10^{-3} ml	2	356	1	466

Strains: The male was W 2324 and the female W 3064. 0.1 ml of twentyfold concentrated cells was added to 0.4 ml minimal medium without glucose, without or with M/2000 $NaIO_4$ (indicated by *), then incubated for 10 minutes at 37°C. Each tube received 4.5 ml penassay broth, and equal volumes of these suspensions were mixed in various combinations and incubated at 37°C for 15 minutes to allow mating. The recombinants were counted by plating on minimal medium containing streptomycin and thiamin.

TABLE 2

EFFECT OF SODIUM PERIODATE ON PAIR FORMATION IN *Escherichia coli*

Per cent survival after treatment	Treated male ♂ 19	Untreated male (100)	Untreated male (Mixture plated immediately)*
	Recombinants on plating		
0.01 ml	16	2,000	40
0.001 ml	0	206	

Strains: The male was W 2324 and the female W 3064. The treatments were similar to the prescription of Table 1. However, the experiment compared male cells that *were* or *were not* exposed to periodate for 15 minutes before mating. The mixture was incubated at 37°C for ten minutes to allow mating pairs to form, then diluted in penassay broth by gentle pipetting; 0.1 ml samples of suitable dilutions were mixed with 5 ml of melted enriched minimal agar and poured on prepared plates of minimal agar. After 5 hours incubation of 37°C, a top layer of 5 ml of minimal agar containing 1,200 μg/ml of dihydrostreptomycin was poured on, the plates were incubated at 37°C for two days, and the large recombinant colonies were counted. Survival after periodate treatment was estimated by viable counts on EMB egar.

* To estimate background of recombinants from matings occurring on the plate without the benefit of earlier pairing.

Viable counts of the treated suspensions were made concurrently on *EMB* agar. The yield of recombinants at the cell densities employed here is proportional to the product of the concentrations of the viable male and female organisms[6]; hence, the expected effect of reduced viability was readily calculated. However, we do not rely on this calculation when the periodate is found to have killed more than 90 per cent of the organisms. The most useful concentration was M/2000 sodium

periodate since this usually reduced the number of matings to about 1 per cent of the normal without killing more than 50 per cent of the treated organisms. It should be noted that the toxicity of $NaIO_4$ was quite sensitive to the temperature and media employed.

Periodate and iodate were assayed as in reference 7. Formaldehyde was detected as aldehyde by the Schiff reaction.

Results.—Inactivation of male cells: E. coli has a considerable capacity for reducing periodate to iodate, and packed organisms will destroy about an equal volume of M/10 $NaIO_4$ in a few minutes. Both iodate and formaldehyde, which may also be formed in the reaction, were inactive at concentrations equivalent to the periodate levels used in tests for toxicity and conjugal inactivation. Periodate is active at 0°C and remains active when it is freshly added to penassay broth, but the concentration must then be increased to compensate for the rapid destruction.

An illustrative experiment is shown in Table 1. It is seen that periodate has killed less than half of the cells but that when male cells were treated before mating, the number of recombinants was greatly reduced. Treatment of female cells has no significant effect, so the periodate does not make female cells infertile.

Recovery of male cells: When periodate-treated male cells were incubated in penassay broth at 37°C, there was no recovery of their virility for 30 minutes; after this, the virility increased and returned to normal after $1^1/_2$ to 2 hours.

Inhibition of F transfer: Treatment with periodate also inhibited the capacity of F^+ male cells to transfer the F agent to F^- cells. Since F^+ cells have relatively low fertility, the transfer of F was studied with a testing system employing the female$_3$ strains described by Richter.[8] The F^+ strain used as F donor (W 6) was streptomycin-sensitive. The F^- strain used as F recipient (W 3086) was streptomycin-resistant. After mixing donor and recipient in penassay for a suitable time, the mixture was plated at a dilution which yielded 50 to 500 colonies per plate onto complete *EMB* medium containing streptomycin. On this medium, only the recipient formed colonies. Next day these colonies were replica-plated onto selective media spread with 0.5 ml of a young broth culture of a ♀$_3$ strain, e.g. W 3876, on *EM* lactose agar. F^+ transfers to cells of W 3086 are detected as colonies which infect the lawn of strain W 3876, converting this to F^+, ♂$_3$, a genotype of very high fertility. The ♂ patch undergoes frequent recombination with adjacent F^- W 3086 to yield M^+ Lac^+ recombinants which grow out conspicuously.

The finding of Jacob and Wollman[9] of a minimum time of about 4 minutes for the contact transmission of the F agent was confirmed. Treatment with periodate of the F donor greatly reduced the amount of F transfer, while treatment of the F recipient had no such effect. Periodate-treated donor cells also failed to transmit the mutant F factors described by Adelberg, et al.[10] and Hirota.[11]

Perborate and persulfate were found to be weakly male-attenuating. Perborate is sometimes found to compete with periodate in chemical combination with carbohydrate. Sodium perborate (M/100) did not block the subsequent male-attenuation by periodate, but rather tended to potentiate it, without, however, increasing the lethal effect.

Other effects of periodate: The "male" mating types of *E. coli* so far described are in fact able to act as weak females. By mating two Hfr_2 male strains, W 3776 and W 3780, for only 15 minutes and selecting for M^+ T^+ L^+ recombinants, one can

study W 3780 as ♂ × W 3776 as ♀, $T^+ L^+$ and not M^+ genes being transferred by Hfr_2 cells in this short time.[12] In this system, periodate-attenuated W 3776 retained its female function.

If a mixture of *Hfr* male and F^- female cells is incubated for a few minutes to allow mating to commence, the orderly transfer of *Hfr* markers is not prevented by the subsequent addition of periodate. Therefore, the periodate does not prevent the continuance of the mating process in those cell pairs which had commenced to mate before periodate was added. This suggests that the action of periodate is to prevent the first step in conjugation, the formation of mating pairs. This possibility was further investigated by the estimation of plate recombinants as indicated in Table 2. In this experiment, those microcolonies derived from a mating pair would contain both strains and these two strains would be able to mate within the microcolony to give streptomycin-resistant prototrophic recombinants. The latter would grow into large colonies, unlike the parental types, and their number would be an indication of the number of mating pairs originally present in the plate. It was found that prior treatment of the male cells with periodate greatly decreased the number of recombinant colonies in this experiment as compared to appropriate controls, thus affording evidence that it had indeed prevented the initial pairing of cells.

The fact that periodate does not interfere with matings which have already commenced should enable one to use this technique to study the kinetics of gene-transfer uncomplicated by subsequent matings. Preliminary experiments showed little difference from matings done without periodate, however, and this suggests that the majority of possible matings in broth in any case take place within a few minutes.

Conclusion and Discussion.—The simplest interpretation of these experiments is that male cells, carrying the *F* particle either in the cytoplasm or on the chromosome, are endowed with a specific conjugal substance on their surface. This substance would then be a periodate-reactive carbohydrate. The oxidation of this carbohydrate would prevent the effective recognition of female cells and formation of conjugal pairs. However, pairs that have once formed are so united that fertilization continues despite the alteration of this specific male substance. This proposal finds some support in the recent discovery by Ørskov and Ørskov[13] of a specific antigen which is regularly associated with the male character in *E. coli*. Attempts to assay this sexual receptor by the blocking power of extracts of male cells on sexual interaction have been inconclusive and further study of this hypothetical substance must await a more suitable assay, perhaps a serological one. No specific substances have so far been found associated with female cells and may be difficult to demonstrate since even male cultures are endowed with some female functional capacity. A more appropriate comparison may perhaps contrast sexually fertile F^- strains with completely sterile F° strains as suggested by Baron *et al.*[14]

The analogy of the sexual receptor to the receptors for influenza virus has already been mentioned as the principal basis for having tested the activity of periodate. Even more striking has been the finding that the mating capacity of one mating type in the yeast *Hansenula wingei* is susceptible to periodate. In this system, unlike *E. coli*, a complementary protein has, furthermore, been demonstrated on the cells of the opposite mating type, this mating type being susceptible to trypsin.[15] A number of enzymes, including snail stomach cytase, trypsin, chymotrypsin,

ribonuclease, and deoxyribonuclease have repeatedly been tested for their effect on mating in *E. coli* without any selective action having been found. The specific sensitivity of male function to periodate therefore remains a solitary clue to the chemical mechanism of conjugation.

The periodate-sensitive structure of male *E. coli* may show some analogy to the acrosome of the animal spermatozoon.[16] At least, the acrosome is reported to contain mucoproteins, stainable with Schiff's reagent after periodate treatment, and is believed to react specifically with the surface materials of the ovum.

Summary.—Periodate, and to a lesser extent, persulfate and perborate, devirilize the male mating types of *E. coli* K-12. The devirilized cells can still act as females, however, and female mating types are not affected by periodate at the relevant concentrations. Devirilized cells recover their virility after about 2 hours in broth culture. The action of periodate appears to be to prevent the formation of mating pairs of cells, probably by altering the surface properties of male cells. The effect is consistent with an oxidation of glycol links in a polysaccharide on the surface of male cells. Treatment of F^+ cells with periodate also makes them unable to transmit the *F* agent by infection.

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[5] The symbols for the genetic markers have the following significance: F^-, standard female mating type; F^+, standard infective male mating type; $♀_3$, female mating type studied by Richter (see below)[8]; Hfr_2, male giving high frequency of recombination, Hayes type; T^-, L^-, Th^-, M^-, growth requirements for threonine, leucine, thiamin, and methionine respectively; Lac^-, Gal^-, Xyl^-, Ara^-, Mal^-, inability to utilize lactose, galactose, xylose, arabinose, and maltose respectively, with subscripts for different loci; S^s, S^r, sensitivity and resistance, respectively, to streptomycin; Lp^s, sensitivity (contra Lp^+ for lysogeny) to phage lambda; V^r_λ, V^r_1, V^r_6, resistance to phages lambda, T_1 and T_6, respectively; Az^r, resistance to azide.

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